2019

The effect of helper presence on egg size in cooperatively breeding birds

BEHAVIOURAL BIOLOGY RESEARCH

NADIA VAN EEKELEN (S3109801)

SUPERVISOR: SIMON VERHULST

Index

Abstract

Cooperative breeders receive help with offspring care from non-breeding individuals, named helpers. In the presence of helpers, parents can reduce their work-load without consequences for their offspring, a phenomenon called load-lightening. Load-lightening at the feeding stage is a common strategy among cooperative breeders. Russell et al. 2017 have shown that for the superb fairy-wren, load-lightening can already occur at the egg stage, in which females that are assisted by helpers lay smaller eggs. They predicted that load-lightening at the egg stage also is a widespread phenomenon among cooperatively breeding species. In this study, this prediction is tested by examining the effect of helper presence on egg size in ten cooperatively breeding bird species: the superb fairy-wren, carrion crow, sociable weaver, brown-headed nuthatch, acorn woodpecker, southern lapwing, Iberian magpie, laughing kookaburra, dunnock and the red-winged fairy-wren. For four of these species, it was demonstrated that females breeding in the presence of helpers laid smaller eggs than in the absence of helpers. In the other six species the effect was the other way around (helper presence increased egg size) or there was no effect at all. Therefore, load-lightening at the egg stage does not appear to be a widespread phenomenon in cooperatively breeding birds. However, there is a chance that the correlation between helper presence and egg size is non-causal, since both egg size and helper presence might be correlated to the quality of the territory and/or breeders. Future studies investigating the role of helper presence in cooperative breeders should take this into account.

1. Introduction

Cooperative breeding is a situation in which non-breeding individuals, named helpers, assist breeders in taking care of the offspring (Paquet et al. 2015). In cooperatively breeding species, offspring receive food from their parents and additional food from the helpers. When the parents maintain the same level of investment in their young in the presence of helpers as they would without helpers, the total care of their offspring increases. However, in many cooperative breeders, helpers do not seem to have an effect on offspring growth and survival. This suggests that the benefits of helpers might be compensated by the parents. That is, with the presence of helpers parents can reduce their effort in offspring care (Hatchwell 1999).

When the activity of the helpers allows parents to reduce their work-load, this is called load-lightening or 'concealed helper effects' (Crick 1992). Load-lightening by breeders, by reducing the feeding rate of the chicks when there are helpers to assist, is commonly observed in cooperative breeders (Hatchwell 1999). It is shown that load-lightening can already occur at the egg stage, resulting in a decreased investment in egg size by mothers breeding in the presence of helpers (Russell et al. 2007). The size of eggs is an important indicator of females' energetic investment in reproduction (Paquet et al. 2013). Producing smaller eggs gives rise to smaller chicks and yields suboptimal offspring, but is beneficial in the presence of helpers. Females breeding in groups can take advantage of the helpers by reducing their own investment in the production of eggs and reallocating the saved energy to future survival and future breeding attempts (Dixit, English, and Lukas 2017; Lejeune et al. 2016).

Conversely, other studies have shown that females increase their investment in reproduction when helpers are present (Russell and Lummaa 2009; Valencia et al. 2016). This is known as the differential allocation hypothesis (Sheldon 2000). According to this hypothesis, females should increase their investment when the current reproductive value is higher than expected. Breeding in the presence of helpers is considered as a situation in which the reproductive value is higher, given that helpers assist in brood provisioning. Therefore, the presence of helpers leads to a situation where females should increase investment in reproduction. Just as with load-lightening, this increase can occur at the egg stage, leading to larger eggs in the presence of helpers (Russell and Lummaa 2009; Valencia et al. 2016; Dixit et al. 2017).

Thus, the load-lightening hypothesis predicts a decrease in egg size when breeding in the presence of helpers, whereas the differential allocation hypothesis predicts an increase in egg size when breeding in the presence of helpers. However, other studies show that egg size increases when more food is available (Koenig et al. 2009; Verhulst et al. 1991). This suggests that the variation in egg size may be caused by the quality of the territory, rather than by the presence of helpers (Russell and Lummaa 2009). A high-quality territory is more likely to attract more helpers (Brouwer, Richardson, and Komdeur 2012), leading to the assumption that the presence of helpers affects egg size, whilst this correlation might be non-causal. In this scenario, there might be no effect found of helper presence on egg size.

Nevertheless, Russell et al. 2007 have found support for the load-lightening hypothesis in superb fairy-wrens (*Malurus cyaneus*), a passerine bird whose social mating system consists of both breeding pairs and breeding groups. Superb fairy-wren mothers reduce their egg investment when breeding in the presence of helpers, by laying eggs that are 5.3% smaller than the eggs from mothers breeding in pairs. Furthermore, they found that the undernourishment of the offspring is completely concealed by the benefits of the helper contributions to offspring care, which confirms the load-lightening hypothesis in this species. In view of this finding, Russell et al. 2007 predict that load-lightening by reducing investment in egg size is a widespread phenomenon in cooperatively breeding birds, although it is not yet been shown in many other species.

1.1 Question and approach

To test this prediction, Dixit et al. 2017 have provided a meta-analysis of a number of studies of in total nine cooperatively breeding bird species and calculated the overall estimated relationship between egg size and the presence of helpers. Their results suggest that in most of these studies, females produce smaller eggs if helpers are present and that across these studies, a significant negative relationship exists between these two variables, a finding that supports the load-lightening hypothesis. However, given that some species substantially produced larger eggs in the presence of helpers, it is unclear whether load-lightening at the egg stage is in fact a common strategy among cooperative breeders.

Here, I will collect and compare studies of cooperatively breeding bird species that provide information of the maternal investment in eggs in the presence of non-breeding helpers. I will examine each cooperatively breeding species separately, provide an overview of the effect sizes given by Dixit et al. 2017 and add more information, in order to determine if the prediction made by Russell et al. 2007 is correct. Hence, my research question is: is load-lightening at the egg stage a common strategy among all cooperatively breeding bird species?

1.2 Methodology

A literature search was conducted to find papers in which maternal investment in egg size and helper presence are studied. As the paper of Russell et al. 2007 inspired this study, I started by looking into the references of this paper and searching for papers in which this study was citated. This brought me to the meta-analysis described in the introduction. By examining the supplemental information for this article I found nine cooperatively breeding bird species and the references of the corresponding papers used in the meta-analysis. I then scanned these papers and their references, and found additional relevant papers on these and other bird species.

Dixit et al. 2017 calculated the effect sizes, the correlation between the two variables: egg size and helper presence. Furthermore, they also calculated the variances in these effect sizes using the following formula: the variance in effect size per analysis unit is $1/(N_j - 3)$, where N_j = sample size for analysis unit (Mcdonald et al. 2016). All data they extracted from the papers and used in the meta-analysis were provided in one table. Relevant information was derived from this table, namely: study, species name, Latin name, measure type (e.g. egg volume or egg mass), sample size, effect size (r), effect size (Z), variance, important notes, and references.

In several studies, the authors did not only report a relationship between egg size and helper presence, but also took into account multiple other relationships. For example differences in the effect of male and female helpers, variation among individual females or within individual females, or age of the mother. In the meta-analysis the effect sizes were calculated for each situation (e.g. egg volume in the presence of male helpers, or egg volume in the presence of female helpers). As I wanted one effect size per species, I took the effect size I thought to be the most relevant for this study.

For the acorn woodpecker, I took the effect size for 'groups with two breeders plus non-breeding helpers.' For the dunnock, I took the effect size for 'egg volume.' For the red-winged fairy-wren, I took the effect size for 'female helpers; comparisons within females,' as male helpers seemed to have no significant effect on egg size. I took the comparison within females rather than between females, because differences in egg size between females can likely be explained by many more variables other than helper presence, for example because a larger female may lay a larger egg (Lejeune et al. 2016).

For the Iberian magpie, I took the effect size for 'mother age not included.' And last, for the laughing kookaburra I took the effect size for 'all helpers.' Furthermore, I added one bird species to the dataset: the brown-headed nuthatch.

I calculated the standard deviation of the effect sizes by taking the square roots of the variances. To visualize the effect sizes I imported my final dataset in Rstudio and conducted a ggplot, depicting species, effect size (Z) and standard deviation. Additionally, for all species of which egg volume/mass was given, both in the presence and in the absence of helpers, or when I was able to calculate them myself using other available data, I conducted a ggplot in Rstudio to display the results in a figure.

2. Helper presence and reduced egg size

2.1 Superb fairy-wren (*Malurus cyaneus*)

As mentioned in the introduction, the phenomenon of concealed helper effects (load-lightening) is reported in superb fairy-wrens by Russell et al. 2007.

To summarize, in the presence of helpers the nestlings receive more food than in the absence of helpers. However, their mass or survival is not influenced by the helpers: the mass of nestlings reared in groups is similar to the mass of nestlings reared by pairs. Consequently, Russell et al. 2007 investigated whether mothers reduced their investment in eggs when breeding in groups, and concluded that females breeding in the presence of helpers lay smaller eggs than females breeding in the absence of helpers (**Figure 1**), leading to helper effects that are not noticeable.

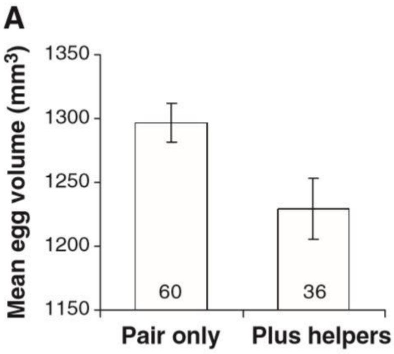


Figure 1*. Relation between helper presence and mean egg volume in the superb fairy-wren. Taken from Russell et al. 2007. In the presence of helpers, females lay smaller eggs than in the absence of helpers (pairs: 1296.91±2.8 mm^3, n=60; groups: 1228.5±5.53 mm^3, n=36).*

2.2 Southern lapwing (*Vanellus chilensis*)

Just as the superb fairy-wrens, the social mating system of southern lapwings consists of breeding pairs and breeding groups. The development of the chicks is precocial, meaning that the birds are hatched in an advanced state and able to feed themselves almost immediately. In this bird species, helpers thus do not feed the chicks, but rather offer protection against predators. Santos and Macedo 2011 tested if females in this situation decrease their investment in eggs (load-lightening) or increase their investment in eggs (differential allocation) when breeding in groups. They found that females breeding in groups have smaller egg sizes than females breeding in pairs.

Santos and Macedo 2011 provide a significant difference between egg volumes from females with helpers and egg volumes from females without helpers. The effect size is -0.23, indicating a negative association between helper presence and egg size (**Figure 2**). Despite the differences in egg size, chicks reared in groups and chicks reared in pairs had a similar mass. These findings support the load-lightening hypothesis. So interesting to see is that, even in a situation where chicks are not fed by helpers, females can allow themselves to reduce their investment in eggs without consequences for their offspring.

A possible reason for this is that the helpers might be spending time leading the chicks to food patches, and that the presence of helpers allows for more frequent forays to these food patches. In this way, the chicks might be able to compensate for the lower maternal investment at the egg stage.

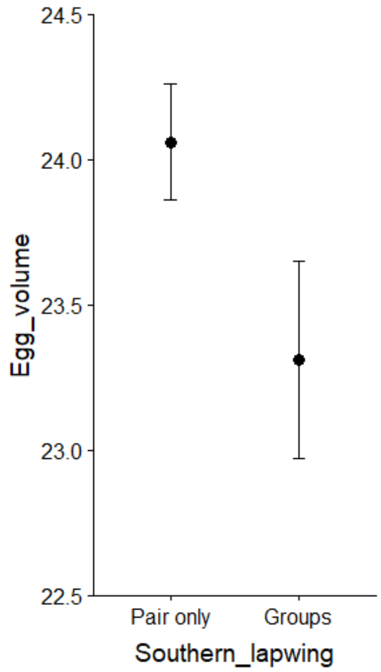
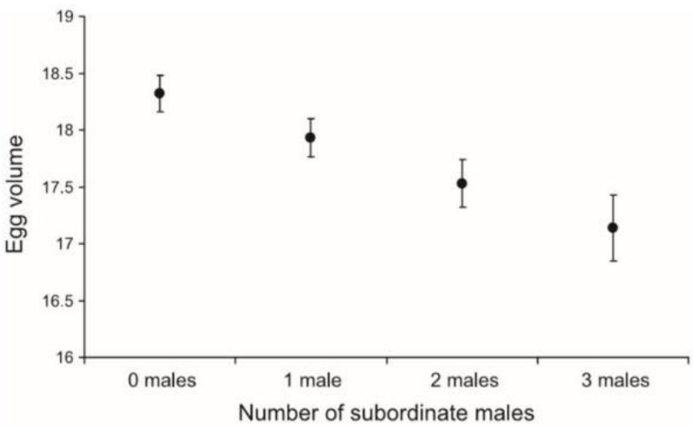


***Figure 2**. Relation between egg volume and helper presence in the southern lapwing. Females breeding in the presence of helpers lay smaller eggs than females breeding without helpers (pairs: 24.06±0.2 cm^3, n=47; groups: 23.31±0.34 cm^3, n=26).*

2.3 Carrion crow (*Corvus corone*)

In cooperatively breeding carrion crows, non-breeding males are most helpful at the nest. Breeding females decrease their egg sizes as the number of helper males increases in the group (Canestrari, Marcos, and Baglione 2011) (**Figure 3**). So in this species, females not only decrease egg size in the presence of helpers, but even decrease it further when the number of helpers increases. In other cooperatively breeding species, a changing number of helpers often has no effect, and it is merely the presence of helpers that influences investment in egg size (see for example Louter et al. 2016). The results from Canestrari et al. 2011 provide support for the load-lightening hypothesis, since the helper effects are hidden by the decreased investment in egg size by females.

***Figure 3**. Relation between helper presence and egg volume in the carrion crow. Taken from Canestrari et al. 2011. Egg volume significantly decreases as the number of male subordinates increases (n=87). With each additional male, egg volume decreases with 2.15%.*



2.4 Sociable weaver (*Philetairus socius*)

Similar to the carrion crow, egg mass significantly decreased with the presence of helpers and the number of helpers (Paquet et al. 2013) (**Figure 4**). As egg mass decreased with group size, the fledging mass of the chicks did not vary. This suggests that the helper effects are concealed by the decreased investment in egg size, again providing support for the load-lightening hypothesis.

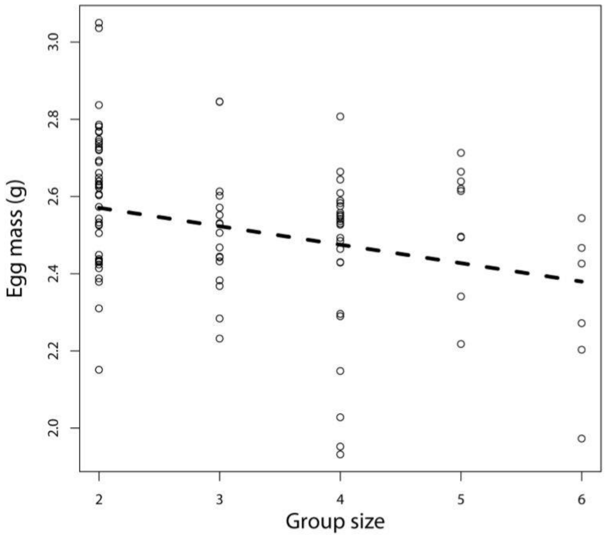


***Figure 4**. Relationship between helper presence and egg mass in the sociable weaver. Taken from Paquet et al. 2013. Egg mass significantly decreases with the presence of helpers and the number of helpers. The number "2" on the x-axis indicates a breeding pair, "3" a breeding pair with one helper, "4" a breeding pair with two helpers, etc. With each additional helper, egg mass decreases with 1.67%.*

3. Helper presence and increased egg size

3.1 Iberian magpie (*Cyanopica cooki*)

Contrary to the previous four bird species, Iberian magpie females seem to invest more in their eggs when helpers are present (Valencia et al. 2016) (**Figure 5**). However, this is only true when the presence of helpers is predictable at the time of egg laying. Included in figure 5 are the so-called 'first-option helpers,' those that do not initiate their own breeding and only assist other's nests. Second-option helpers (not included in the figure) are those that first attempt their own breeding, but assist other's nests as soon as their own fails. First-option helpers are thus more predictable and their presence has a positive effect on the egg sizes of breeding females. Therefore, the findings concerning the first-option helpers provide support for the differential allocation hypothesis.

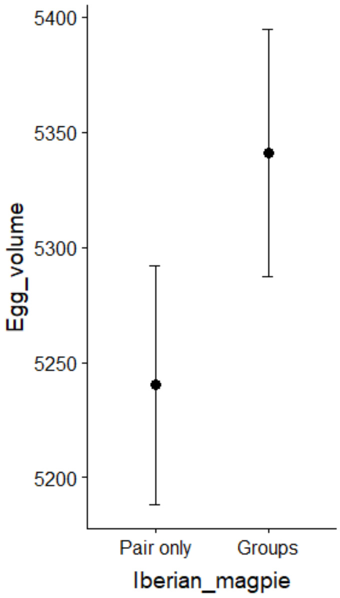


***Figure 5**. Relation between helper presence and egg volume in the Iberian magpie. Females breeding in the presence of first-option helpers lay larger eggs than females breeding without first-option helpers (pairs: 5341.06±53.9075 mm^3, n=31; groups: 5240.28±51.955 mm^3, n=85).*

However, when the second-option helpers are also taken into account, the effect fades and the presence of helpers is not associated with an increased egg size. Likely, the cause for not altering egg size in the presence of helpers thus is that the predictability of helper presence is low at the egg stage. Most helpers in the Iberian magpie do not assist before the nestlings hatch.

Still, according to the authors, a pattern emerges towards more investment in eggs when helpers are present. This positive effect between egg size and helper presence might also be because a large role of helpers in the Iberian magpie is to reduce predation risk. According to the theoretical model by Carranza et al. 2008, based on the azure-winged magpie (*Cyanopica cyanus*), parents should increase their investment in such a situation.

3.2 Laughing kookaburra (*Dacelo novaeguineae)*

The Iberian magpie is not the only cooperative breeding species that tends to increase egg size in the presence of helpers. Laughing kookaburra helpers appear to show the same effect on maternal investment in egg size (Legge 2000a; Legge 2000b), although the effect is small. Female helpers in this species are relatively poor helpers (Legge 2000b). So, producing slightly larger eggs when there are more female helpers than male helpers might be the solution to the limited help they receive from females after the hatching of the offspring. Furthermore, laughing kookaburra's already prefer to reduce their workload during breeding. Thus, the costs of raising offspring are already high (Legge 2000a) and breeding females possibly cannot reduce their investment further at the egg stage than they already do.

The results provide no support for the load-lightening hypothesis, but also no support for the differential allocation hypothesis, since this suggests that females should take advantage of the favourable conditions of helpers being present. However, for the laughing kookaburra, helpers mostly have a neutral effect or even a negative effect. A possible reason for why females lay larger eggs in the presence of helpers can be because for this species, territories with a high number of helpers might be poor compared to territories with a small number of helpers. In a poor-quality territory there is less food available to rear the chicks and it might thus be beneficial to lay larger eggs.

4. No effect of helper presence on egg size

4.1 Acorn woodpecker (*Melanerpes formicivorus*)

Koenig et al. 2009 shows that there seem to be no significant differences in egg size in relation to helper presence in the acorn woodpecker. Furthermore, egg size is not adjusted in a way that conceals the effects of helpers (Koenig, Walters, and Haydock 2009; Legge 2000a). These findings thus support neither the load-lightening hypothesis, nor the differential allocation hypothesis.

Figure 6*. Relation between mean egg volume, helper presence (male: left; female: right) and acorn crop (divided in good and poor years) in the acorn woodpecker. Taken from Koenig et al. 2009. Females breeding in the presence of helpers lay smaller eggs in poor acorn years.*

Nevertheless, the results from Koenig et al. 2009 demonstrate that egg size varies depending on several other variables different from helper presence. They suggest that the maternal adaptive adjustment of egg size is related to ecological conditions. These ecological conditions include environmental temperatures and food supply (acorn crop, a critical food source for this species (Koenig and Mumme 1987)). When temperatures were warm prior to egg-laying, acorn woodpecker eggs were significantly larger in volume. Furthermore, the acorn woodpecker females lay smaller eggs when they are assisted by helpers in poor acorn years and larger eggs in good acorn years (**Figure 6**). In the figure above, a difference is made between the effects of male helpers and female helpers. The pattern observed is identical, however, the result is significant for female helpers but not for male helpers.

A possible reason for why there is no effect of only helper presence on egg size could be that, just as with the Iberian magpie, females are unable to predict the presence of helpers (Valencia et al. 2016). However, this is likely not the case for acorn woodpeckers, since helpers are generally offspring from previous years (Koenig et al. 2009). The predictability of helpers being present is thus quite large.

4.2 Brown-headed nuthatch (*Sitta pusilla*)

Just as for the acorn woodpecker, there is no effect of the presence of helpers on egg sizes of the brown-headed nuthatch. The volume of eggs laid by females in the presence of helpers is similar to the volume of eggs laid by females in the absence of helpers (Cusick et al. 2018) (**Figure 7**).

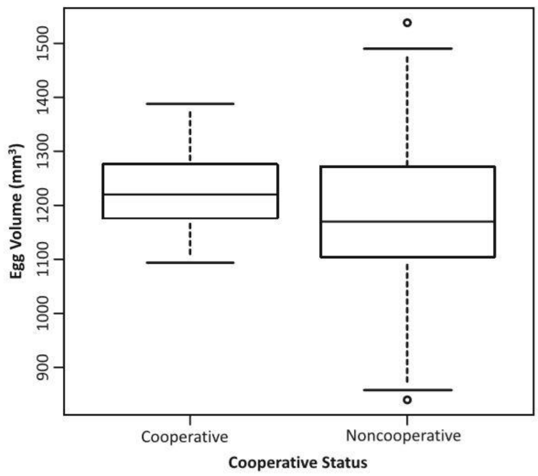


Figure 7. *Relation between helper presence and egg volume in the brown-headed nuthatch. Taken from Cusick et al. 2018. Cooperative = helpers are present; noncooperative: helpers are absent. Egg volume is unrelated to helper presence. There is no significant difference between the volumes of eggs laid by females assisted by helpers (1225.4±10.7 mm^3, n=42) and the volumes of eggs laid by females without helpers (1188.3 ±10.9 mm^3, n=472).*

A possible reason is that brown-headed nuthatch helpers do not assist in nest excavation. Nest excavation is typical behaviour for this species. It is one of the early stages of reproduction and can take up to weeks to complete (Cusick et al. 2018). If helpers accelerate the excavation, it could have an effect on the future investment decisions that are made by breeders, especially since nests that are finished earlier are more successful (Carranza et al. 2008; Cusick et al. 2018). However, the helpers do not contribute to nest excavation and are usually present from the moment the chicks hatch. According to Cusick et al. 2018, the predictability of helper presence is therefore low. Due to this low predictability, females are not likely to change their investment in eggs, resulting in similar egg volumes between the cooperative and noncooperative groups.

4.3 Dunnock (*Prunella modularis*)

Within populations, dunnocks show variable mating systems. Breeding groups can range from monogamy, polyandry, polygyny and polygynandry. To study the effect of helpers on egg size, a population consisting of monogamous and polyandrous (one female with two or three males) groups is investigated. There is no difference in the volume of eggs laid by females without helpers (monogamous/pair only) and females with helpers (polyandrous/groups) (Santos and Nakagawa 2013) (**Figure 8**).

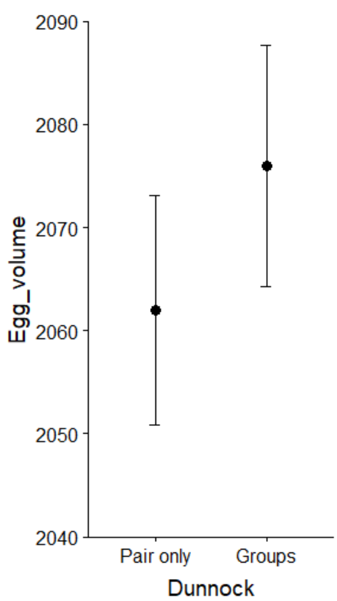


Figure 8*. Relation between helper presence and egg volume in the dunnock. There is no significant difference in the volume of eggs laid by females without helpers and females with helpers (pairs (monogamous): 2062±11.17 mm^3, n=229; groups (polyandrous): 2076±11.73 mm^3, n=198).*

Females from these two breeding strategies show similar investment in the egg stage, and do not change this when expecting the assistance of helpers. Consequently, these findings do not support both load-lightening and differential allocation.

For the brown-headed nuthatch, the low predictability of helper presence is likely the reason for why there is no effect of helper presence on egg size. However, for the dunnock the predictability of helper presence is high, since individuals usually do not switch groups during the breeding season. Therefore, this is likely not the reason and the actual reason is yet to be determined.

Another study on dunnocks, by Davies and Hatchwell 1990, examined what would happen to female provisioning if their help was reduced from two males to one, to one male's part-time help or to no male help at all. So instead of examining the effect of the helper presence on egg size, the question was what would happen if the number of helpers decreases. They found that female provisioning stayed the same when help was reduced from two males full-time to one male full-time, but that it did increase when help was further reduced to one male part-time or no male help. The increase of investment takes place at the feeding stage, however, this finding might suggest that this phenomenon can also occur at the egg stage. Thus, if the latter is true, dunnock females would increase their egg investment when help is reduced, whereas the presence of helpers per se has no effect on egg size.

4.4 Red-winged fairy-wren (*Malurus elegans*)

Another cooperative breeder in which helper presence has no effect on egg size is the red-winged fairy-wren. There is no evidence that red-winged fairy-wren females load-lighten at the egg stage (Lejeune et al. 2016). For the red-winged fairy-wrens, helper effects vary with the sex of the helpers. Groups with more female helpers, and not male helpers, tended to have smaller eggs. Egg size was not decreased in response to a change in the number of both male and female helpers (**Figure 9**). The reason why female breeders lay smaller eggs in the presence of female helpers and not male helpers is still unknown, but a possible explanation is that it might be a result from sex ratio biases, as females hatch from smaller eggs. This remains to be investigated.

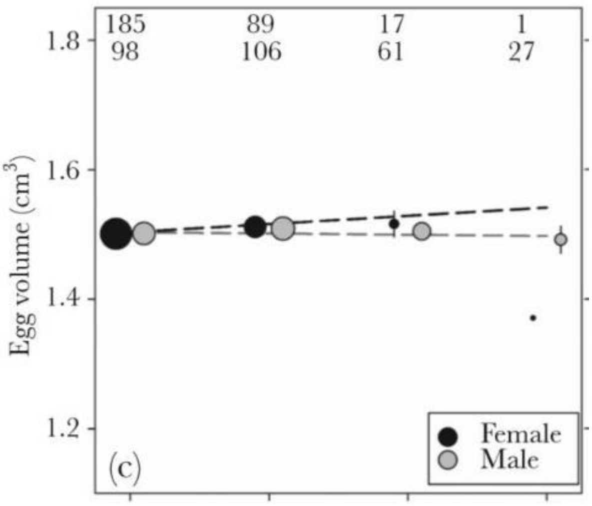


***Figure 9**. Relation between the number of female (black) and male (grey) helpers for egg volume in the red-winged fairy-wren. Taken from Lejeune et al. 2016. The numbers above indicate the sample sizes for the number of female helpers (upper) and male helpers (lower). The symbol sizes are adjusted according to the sample sizes.*

It is interesting to see a large difference between the red-winged fairy-wren and the superb fairy-wren, since these species are so closely related. The difference might be explained by the predictability of helper presence. In the red-winged fairy-wren, female helpers are much more common than in the superb fairy-wren. Female helpers are less reliable than male helpers, and that lower reliability might explain the absence of load-lightening at the egg stage (Brouwer, Van de Pol, and Cockburn 2014; Lejeune et al. 2016). The reason female helpers are less reliable than male helpers, is because female helpers can also initiate their own nest and because in this species, females disperse earlier than males. Therefore, the predictability of the presence and the reliability of female helpers is low and it might thus be too risky for breeders to modify the investment in eggs.

5. Effect size: helper presence and egg size

To examine the overall relationship between helper presence and egg size for the ten species described, the correlations between these two variables were calculated and/or extracted from publications (**Table 1** and **Figure 10**). All effect sizes come from the meta-analysis conducted by Dixit et al. 2017, except for the brown-headed nuthatch. An effect size of r<0.10 is considered "not significant", 0.10-0.30 "small," 0.30-0.50 "medium," and >0.50 "large" (Cohen 1988).

To find the overall effect of helper presence on egg size in these species, the weighted average was calculated by the following formula: $\frac{n1r1 + n2r2 + n3r3 + \dots}{n1 + n2 + n3 + \cdots}$. Resulting from this formula is a mean effect size of -0.14345. Because this is negative, it indicates that egg sizes are more likely to be reduced in the presence of helpers. However, the effect is considered small.

***Table 1**. Effect sizes, variances, sample sizes and standard deviations of ten cooperatively breeding bird species. A negative effect size indicates a smaller egg size in the presence of helpers. A positive effect size indicates a larger egg size in the presence of helpers. * indicates for which species effect sizes are not taken from Dixit et al. 2017.*

Species	r	Z	Variance	Standard deviation	n
Carrion crow	-0.677048654	-0.823644546	0.011904762	0.109108946	87
Sociable weaver	-0.416241096	-0.443136732	0.004016064	0.063372423	252
Superb fairy-wren	-0.229667224	-0.233838132	0.010638298	0.103142125	96
Brown-headed nuthatch*	-0.16300000	-0.164467041	0.001956947	0.044237395	514
Acorn woodpecker	-0.156362521	-0.157655863	0.005181347	0.071981574	196
Southern lapwing	-0.090182467	-0.090428148	0.014084507	0.118678166	73
Iberian magpie	-0.02820346	-0.028210942	0.003717472	0.060971075	272
Dunnock	0.026246322	0.026252351	0.008264463	0.090909092	124
Red-winged fairy-wren	0.038374506	0.03839336	0.003460208	0.058823533	292
Laughing kookaburra	0.232025689	0.236329356	0.014705882	0.121267811	71

In **Table 1** the effect sizes are shown for each cooperatively breeding species. To know if the effect is considered large, medium, small or non-significant we look at the effect size r, shown in the first column. The effect size for the carrion crow is considerably large. The effect size for the sociable weaver is moderate. The effect sizes of the superb fairy-wren, brown-headed nuthatch, acorn woodpecker, and laughing kookaburra are small and the effect sizes of the southern lapwing, Iberian magpie, red-winged fairy-wren and dunnock are not significant.

Unfortunately, not all effect sizes calculated by Dixit et al. 2017 seem to agree with the results described in the corresponding papers. Because most data used to calculate the effect sizes is not available in these papers, but were obtained by Dixit et al. 2017 by personal communications with the authors, it was not possible to calculate them again. For the southern lapwing, the effect size from the meta-analysis is considered not-significant, whereas Santos and Macedo 2011 do provide a significant difference between egg volumes from females with helpers and egg volumes from females without helpers. And contrary to the negative effect size used in the meta-analysis, the Iberian magpie seems to invest more in eggs when helpers are present, although this is merely true for the presence of first-option helpers. The effect fades when second-option helpers are also taken into account, but will not become negative. Then, for the acorn woodpecker, the effect size used by Dixit et al. 2017 suggests females decrease egg size when breeding in the presence of helpers. However, Koenig et al. 2009 shows that there seem to be no significant differences in egg size in relation to helper presence. Last, the small effect size of the brown-headed nuthatch a is also negative, however, egg volumes between the cooperative and noncooperative groups were similar.

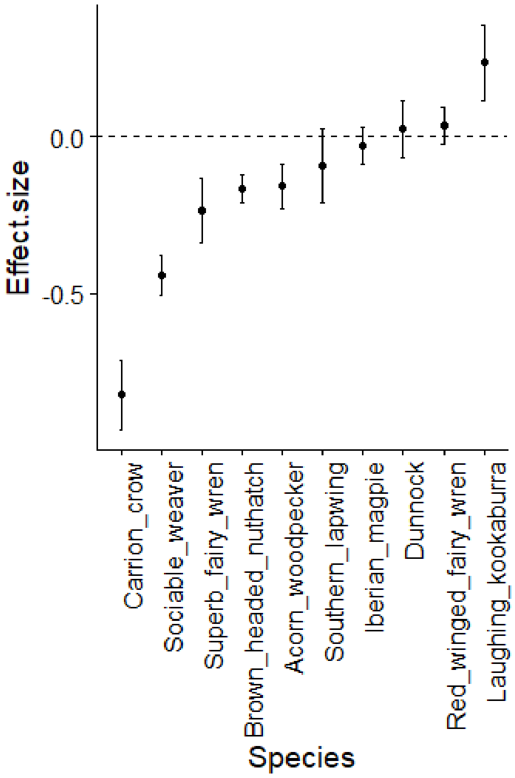


Figure 10. *Effect sizes (Z) of ten cooperatively breeding bird species. On the x-axis: species. On the y-axis: effect size. Points under zero mean egg size is smaller in the presence of helpers. Points above zero mean egg size is larger in the presence of helpers.*

6. Discussion

Russell et al. 2007 were the first to connect the presence of helpers in a group to a reduced maternal investment in eggs. With the study on superb fairy-wrens, they demonstrated that load-lightening can already occur at the egg stage. Consequently, studies on other cooperatively breeding birds also found support for load-lightening (Canestrari et al. 2011; Paquet et al. 2013). For both the carrion crow and the sociable weaver, egg size significantly decreased with the presence of helpers and with an increasing number of helpers. Furthermore, the results from the southern lapwing also strongly support the findings of Russell et al. 2007, but in an unique context: for a cooperatively breeding species with precocial chick development (Santos and Macedo 2011). The southern lapwing helpers do not feed the chicks, but instead offer protection from predators and lead the chicks to food patches. In this way, the reduction of maternal egg investment is compensated, supporting the load-lightening hypothesis.

Interesting to see is that for both the southern lapwing and the Iberian magpie the main role of the helpers is to offer protection from predators. However, the southern lapwing females decrease egg size in the presence of helpers and the Iberian magpie females lean more towards increasing egg size in the presence of helpers. Shortly described in 3.1 is the theoretical model by Carranza et al. 2008. According to this model, parents should increase their investment in a situation where helpers are mainly present to offer protection. This seems to be true for the Iberian magpie, but not for the southern lapwing. A possible reason why the theoretical model does not apply for the southern lapwing is because included in the model are also the effects of food provisioning. Southern lapwing helpers do not feed the chicks, since they are able to feed themselves (Santos and Macedo 2011). Another reason for the difference might be because the predictability of helpers for the southern lapwings is really high, whereas for the Iberian magpie the predictability is low. For the southern lapwing, 72% of the groups are composed of the same individuals and 92% of the groups maintain the same number of individuals over multiple breeding seasons (Saracura, Macedo, and Blomqvist 2008).

Besides the Iberian magpie, the laughing kookaburra also tends to increase egg size in the presence of helpers (Dixit et al. 2017). However, only the data from the meta-analysis concerning the egg size of the laughing kookaburra was available, since these were not mentioned in the corresponding paper but were personally communicated to Dixit et al. 2017. Therefore, the reasons why laughing kookaburra females increase egg size (described in 3.2) are merely speculations. Evidence for a positive effect of helper presence on egg size thus seems to be weak. Worth mentioning is the chestnut-crowned babbler (*Pomatostomus ruficeps*), a cooperative breeder with a social mating system consisting of monogamous pairs and subordinate helpers. Chestnut-crowned babbler mothers increase their investment in eggs when they are in the presence of the optimal number of helpers. The females lay 4% larger eggs in the presence of helpers compared with no helpers. These larger eggs produce chicks that are heavier at hatching and heavier at fledging (Russell and Lummaa 2009; A.F. Russell 2008, unpublished results). The results from the chestnut-crowned babbler show stronger evidence for increased maternal egg investment in the presence of helpers and are consistent with the differential allocation hypothesis (Sheldon 2000), since females reproduce maximally in the presence of helpers. A possible reason for why mothers not reduce, but increase their investment in eggs in the presence of helpers can be because their probability of future reproduction might be low (Russell and Lummaa 2009; Valencia et al. 2016).

Discussed are the cooperatively breeding bird species that modify egg sizes in response to helper presence. However, as shown in paragraph 4, there is also a number of cooperative breeders that do not alter egg sizes when breeding in the presence or in the absence of helpers: the acorn woodpecker, brown-headed nuthatch, dunnock and red-winged fairy-wren (Koenig et al. 2009; Cusick et al. 2018; Santos and Nakagawa 2013; and Lejeune et al. 2016).

As mentioned in paragraph 4.3, a study on dunnocks by Davies and Hatchwell 1990 examined what would happen to female provisioning if their help was reduced instead of increased. They found that female investment increased when help was reduced to part-time help or to no help at all. This same kind of response is found in the pied flycatcher, of which the social mating system consists of monogamy and polygyny (one male with two females). Within polygyny, the male leaves its nest as soon as the female has laid eggs and creates a second territory to attract another female. Even when the male succeeds to breed with the second female, he usually returns to the first female to provide help with offspring care. As the male left the second female, she increases her provisioning rate to the young (Alatalo et al. 1988; Davies and Hatchwell 1990). This finding might indicate that the effect of helper presence on egg size also works the other way around, which leads to the prediction that when help is minimized to part-time help or to no help at all, female breeders should increase their investment at the egg stage. Furthermore, this suggests that species in which females breed polygynously can also be used to test the load-lightening and differential allocation hypotheses.

So, for the last four cooperatively breeding species, helper presence had no effect on egg size. However, that does not take away the fact that the presence of helpers is still beneficial for parameters other than egg size, such as clutch size, hatching success and fledging success. Offspring from the brown-headed nuthatch that are raised by cooperative groups are provisioned more frequently, have a higher mass and higher change of fledging, compared to offspring raised by pairs (e.g. Cusick et al. 2018). More helpers allow red-winged fairy-wren females to lay larger clutches and to reduce re-nesting intervals (e.g. Lejeune et al. 2016). Dunnock chicks reared in polyandrous nests hatched and fledged more than chicks in monogamous nests (Santos and Nakagawa 2013). For carrion crows, offspring raised in groups with more male helpers had a higher body mass at fledging and a higher probability of surviving in the first month (Canestrari et al. 2011). Group size in laughing kookaburra's is positively correlated with fledging weight and success, an indicator of juvenile survival (Legge 2000b). Acorn woodpecker helpers correlate with increased reproductive success (Koenig et al. 2009). Another example is the cooperatively breeding apostlebird (*Struthidea cinerea*). In this species, helpers enhance multiple components of reproductive success, such as annual fledging success, the probability of re-nesting, and provisioning rate per chick (Woxvold and Magrath 2005). Overall, having helpers around to assist with offspring care is advantageous.

It is important to mention that egg size may be correlated to territorial and/or parental quality (Krist 2009). Independently, these can affect egg size and offspring survival. Also, high-quality breeders might live in high-quality territories and produce high-quality offspring, which survive better. A high-quality territory or breeder is more likely to attract more helpers (Brouwer, Richardson, and Komdeur 2012). Therefore, the correlation between helper presence and egg size might be non-causal, and the cause of altered egg size might be the quality of the territory and/or the breeders. To solve this problem, the causality of helper effects can be determined by a comparison of the same group with and without helpers (e.g. Legge 2000b). However, it should be taken into account that changes in helper number are often the result of high reproduction or low survival, which also can be an effect from the quality of the territory.

Further studies are needed to identify the effects of a higher-quality territory/breeder on egg size in the cooperatively breeding species, and to assess if other ecological conditions such as temperature and food abundance affect maternal egg investment in other species besides the acorn woodpecker. Furthermore, the effect of the sex of the helper remains studied poorly; yet, this seems to be an important variable, for example in the red-winged fairy-wren, acorn woodpecker and laughing kookaburra.

7. Conclusion

Finally, load-lightening by reducing maternal investment at the egg stage does not appear to be a widespread phenomenon in cooperatively breeding birds, as Russell et al. 2007 predicted. The overall effect of helper presence on egg size seems to be negative, however, this effect is weak. Whereas egg size is demonstrated to be decreased with helper presence for the superb fairy-wren, southern lapwing, carrion crow and sociable weaver, this is not the case in the six other cooperatively breeding species. Additionally, helper presence is most likely not the only factor affecting egg size, and this should thus be controlled for. Consequently, I consider it unlikely that reducing egg size to adjust parental investment in the presence of helpers (load-lightening) is a common strategy among all cooperative breeders.

8. References

Alatalo R.V., Gottlander K, Lundberg A. 1988. "Conflict or cooperation between parents feeding nestlings in the pied flycatcher *Fieedula hypoleuca* (Pallas)." *JSTOR,* Ornis Scand 9:31-34.

Brouwer, L., Van de Pol, M. and Cockburn, A. 2014. "The Role of Social Environment on Parental Care: Offspring Benefit More from the Presence of Female than Male Helpers." *Journal of Animal Ecology* 83(2):491–503.

Brouwer, L., Richardson, D.S. and Komdeur, J. 2012. "Helpers at the Nest Improve Late-Life Offspring Performance: Evidence from a Long-Term Study and a Cross-Foster Experiment." *PLoS ONE* 7(4):16–20.

Canestrari, D., Marcos, J. M., and Baglione, V. 2011. "Helpers at the Nest Compensate for Reduced Maternal Investment in Egg Size in Carrion Crows." *Journal of Evolutionary Biology* 24(9):1870–78.

Carranza, J., Mateos, C., De la Cruz, C., Valencia, J. and Polo, V. 2008. "How Should Breeders React When Aided by Helpers?" *Animal Behaviour* 75(4):1535–42.

Cohen, J. 1988. *Statistical Power Analysis for the Behavioral Sciences*. Second. New York: Lawrence Erlbaum Associates.

Crick, H. Q. P. 1992. "Load-lightening in Cooperatively Breeding Birds and the Cost of Reproduction." *Ibis* 134(1):56–61.

Cusick, J. A., De Villa, M., DuVal, E.H. and Cox, J.A. 2018. "How Do Helpers Help? Helper Contributions throughout the Nesting Cycle in the Cooperatively Breeding Brown-Headed Nuthatch." *Behavioral Ecology and Sociobiology* 72(3).

Davies, N.B. and Hatchwell, B.J. 1990. "Provisioning of Nestlings by Dunnocks, Prunella Modularis, in Pairs and Trios Compensation Reactions by Males and Females." *Behavioral Ecology and Sociobiology* 27(3):199–209.

Dixit, T., English, S. and Lukas, D. 2017. "The Relationship between Egg Size and Helper Number in Cooperative Breeders: A Meta-Analysis across Species." *PeerJ* 5:e4028.

Hatchwell, B.J. 1999. "Investment Strategies of Breeders in Avian Cooperative Breeding Systems." *The American Naturalist* 154(2):205–19.

Koenig, W.D., Walters, E.J. and Haydock, J. 2009. "Helpers and Egg Investment in the Cooperatively Breeding Acorn Woodpecker: Testing the Concealed Helper Effects Hypothesis." *Behavioral Ecology and Sociobiology* 63(11):1659–65.

Krist, M. 2009. "Short- and Long-Term Effects of Egg Size and Feeding Frequency on Offspring Quality in the Collared Flycatcher (Ficedula Albicollis)." *Journal of Animal Ecology* 78(5):907–18.

Legge, S. 2000a. "Helper Contributions in the Cooperatively Breeding Laughing Kookaburra: Feeding Young Is No Laughing Matter." *Animal Behaviour* 59(5):1009–18.

Legge, S. 2000b. "The Effect of Helpers on Reproductive Success the Laughing Kookaburra." *Journal of Animal Ecology* 69(4):714–24.

Lejeune, L., Van De Pol, M., Cockburn, A. Louter, M. and Brouwer, L. 2016. "Male and Female Helper Effects on Maternal Investment and Adult Survival in Red-Winged Fairy-Wrens." *Behavioral Ecology* 27:arw121.

Mcdonald, C., Rice, D.C., Canto, A.I., Southerland, S.A., Underwood, P., Kaya, S., Fishman, B. and Morrison, F.J. 2016. "The University of Chicago Press." 113(1):52–75.

Paquet, M., Covas, R., Chastel, O., Parenteau, C. and Doutrelant, C. 2013. "Maternal Effects in Relation to Helper Presence in the Cooperatively Breeding Sociable Weaver." *PLoS ONE* 8(3).

Paquet, M., Doutrelant, C., Hatchwell, B.J., Spottiswoode, C.N. and Covas, R. 2015. "Antagonistic Effect of Helpers on Breeding Male and Female Survival in a Cooperatively Breeding Bird." *Journal of Animal Ecology* 84(5):1354–62.

Russell, A. F., Langmore, N.E., Cockburn, A., Astheimer, L.B. and Kilner, R.M. 2007. "Reduced Egg Investment Can Conceal Helper Effects in Cooperatively Breeding Birds." *Science* 317(August):941–44.

Russell, A.F. and Lummaa, V. 2009. "Maternal Effects in Cooperative Breeders: From Hymenopterans

to Humans.” *Philosophical Transactions of the Royal Society B: Biological Sciences* 364(1520):1143–67.
Santos, E.S.A. and Macedo, R.H. 2011. “Load Lightening in Southern Lapwings: Group-Living Mothers Lay Smaller Eggs than Pair-Living Mothers.” *Ethology* 117(6):547–55.
Santos, E.S.A. and Nakagawa, S. 2013. “Breeding Biology and Variable Mating System of a Population of Introduced Dunnocks (Prunella Modularis) in New Zealand.” *PLoS ONE* 8(7):1–7.
Saracura, V., Macedo, R.H. and Blomqvist, D. 2008. “Genetic Parentage and Variable Social Structure in Breeding Southern Lapwings.” *The Condor* 110(3):554–58.
Sheldon, B.C. 2000. “Differential Allocation: Tests, Mechanisms and Implications.” *Trends in Ecology and Evolution* 15(10):397–402.
Verhulst, S. and Tinbergen, J.M. 1991. "Experimental Evidence for a Causal Relationship Between Timing and Success of Reproduction in the Great Tit *Parus M. Major." Journal of Animal Ecology* 60: 269-282.
Valencia, J., Mateos, C., De la Cruz, C. and Carranza, J. 2016. “Maternal Allocation in Eggs When Counting on Helpers in a Cooperatively Breeding Bird.” *Journal of Avian Biology* 48(4):536–43.
Woxvold, I.A. and Magrath, M.J.L. 2005. “Helping Enhances Multiple Components of Reproductive Success in the Cooperatively Breeding Apostlebird.” *Journal of Animal Ecology* 74(6):1039–50.